# Age-Dependent Variation in Mating Success of Sterile Male Mediterranean Fruit Flies (Diptera: Tephritidae): Implications for Sterile Insect Technique

TODD E. SHELLY,[1,2,3] JAMES EDU,[1] AND ELAINE PAHIO[1]

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**ABSTRACT** The sterile insect technique (SIT) is widely used in integrated programs against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Unfortunately, the mass-rearing procedures inherent to the SIT often lead to a reduction in the mating ability of the released males. To counter this deficiency, SIT programs rely upon the production and release of large numbers of sterile males to achieve high overflooding (sterile:wild male) ratios. To ensure a high release volume, emergence facilities release adult males at a young age (2 d old in some cases). The primary objective of this study was to describe age-dependent variation in the mating propensity and competitiveness of sterile males of *C. capitata*. Males that were 2 or 3 d old had lower mating propensity than males that were ≥4 d old, and 3-d-old males had lower mating competitiveness than males that were ≥4 d old. Given these results, we measured the effect of a longer holding period on male mortality in storage boxes. With delayed food placement, males held in storage boxes for 4 d after emergence showed no higher mortality than males held for only 2 d (the standard interval). Using large field enclosures, we compared the levels of egg sterility attained via releases of 2- versus 4-d-old sterile males at two overflooding ratios (5:1 and 100:1). At the lower ratio, the proportion of unhatched eggs observed for trials involving 2-d-old sterile males was not, on average, significantly higher than that observed for matings between wild flies (33 versus 25%, respectively), whereas the level of egg sterility observed for releases of 4 d old sterile males was 62%. At the 100:1 overflooding ratio, the proportion of unhatched eggs associated with the 2-d-old sterile males was 58%, a level not significantly different from that induced by 4-d-old sterile males at the 5:1 ratio and significantly lower than the level (79%) observed for 4-d-old sterile males at 100:1 overflooding ratio. The implications of these results for SIT are discussed.

**KEY WORDS** Mediterranean fruit fly, *Ceratitis capitata*, sterile insect technique, mating success, male age

The sterile insect technique (SIT) is widely used to suppress or eradicate infestations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Hendrichs et al. 2002, Klassen 2005). In an SIT program, males are mass produced (female embryos are selectively killed by heating in genetic sexing strains containing a temperature-sensitive lethal [*tsl*] mutation; Franz et al. 1996), sterilized (with gamma irradiation), and released into the environment to mate with wild females, resulting in infertile eggs. Unfortunately, the mass-rearing environment, particularly the high density at which adults are held, imposes strong artificial selection that may alter male courtship behavior and subsequently lessen the mating competitiveness of sterile males in the wild (McInnis et al. 1996, Lance et al. 2000). To counter this deficiency, SIT programs against *C. capitata* rely upon the production and release of large numbers of sterile males to achieve high overflooding ratios (sterile:wild males). The interplay between fly performance and production is perhaps the single most important factor shaping current implementation of SIT against this pest. The drive to provide "quantitative compensation" for a "qualitatively poor" product has, for example, been partially responsible for the continued expansion of the largest mass-rearing facility in the world (El Pino, Guatemala), which will soon produce ≥3 billion sterile males of *C. capitata* per week.

The perceived need for high overflooding ratios affects not only the rearing facilities themselves but also the processing of sterile flies at the eclosion facilities. Production facilities are typically located far from the release areas, and sterile males are shipped as pupae to eclosion facilities, which are located near or within release areas. Adult emergence occurs at the eclosion facilities, and males are held for several days before release. The protocol followed at the David

[1] USDA–APHIS, 41-650 Ahiki St., Waimanalo, HI 96795.
[2] Center for Conservation Research and Training, University of Hawaii, Honolulu, HI 96822.
[3] Corresponding author, e-mail: todd.e.shelly@aphis.usda.gov.

Rumsey Sterile Fruit Fly Eclosion Facility, Mediterranean Fruit Fly Exclusion Program, in Los Alamitos, CA, is typical of such operations. Irradiated pupae arrive daily on flights from Guatemala and Hawaii and are placed in large, plastic boxes. Adult food (sugar agar) also is placed on the screened opening on the lid of each box at this time. Peak emergence of the adult males occurs 2 d later, and males are released 2 d after peak emergence. Thus, flies from a given (daily) shipment are held for 5 d at this facility: pupae arrive and are placed in the holding boxes on day 1, peak emergence of adults occurs on day 3, and the flies are cooled and released into the environment on day 5 when they are 2 d old.

Although mass rearing has led to accelerated sexual maturation in the Mediterranean fruit fly (Cayol 2000), preliminary observations in our laboratory indicated that most 2-d-old sterile males do not mate with sexually mature, virgin females. Thus, it seems that, regarding the California program, relatively few sterile males are sexually mature when released, and several days may elapse before released males seek mating aggregations, produce sex pheromone (for long-range mate attraction), and attempt to mate. Two main factors promote the release of immature males. Most importantly, the goal of achieving a high overflooding ratio requires the early release of males, because this speeds the flow rate of males through the eclosion facility, which, in turn, more quickly frees resources and space for newly arriving pupae. In addition, as the quality of the sugar agar deteriorates (via desiccation), mortality in the boxes may increase sharply through time, thus favoring early release of the sterile males.

The primary objective of this study was to examine age-dependent variation in the mating behavior of sterile males of *C. capitata.* Initially, three different experiments were performed. First, we monitored the mating propensity of sterile males of different ages in a noncompetitive environment in the laboratory. Second, we measured the mating success of sterile males of differing ages in competition with mature, wild-like males for copulations with mature, virgin, wild-like females in field cages. Third, we monitored the ability of sterile males of differing ages to inhibit remating by wild-like females in both laboratory and field cages.

As a corollary to the mating studies, we examined the effect, in terms of increased mortality, of holding sterile males for longer-than-normal periods before release. In particular, we compared the number of males exiting storage boxes 2 and 4 d after peak emergence with routine food placement (i.e., food placement coincident with pupal placement) to determine the cost (in terms of lowered release numbers) associated with holding males for two extra days. In addition, we estimated the number of exiting males 4 d after peak emergence with delayed food placement (i.e., food placement coincident with the day of peak emergence), expecting that this treatment would reduce mortality relative to that observed for the 4 d postemergence treatment with routine food placement.

A final experiment was conducted that incorporated the results of the aforementioned mating and mortality studies. As will be shown, sterile males that were $\geq$4 d old displayed higher mating competitiveness than 2-d-old sterile males, and delayed placement of food on the storage boxes (to coincide with peak emergence) reduced mortality among males held in the boxes for 4 d. Based on these findings, we conducted a study comparing the effectiveness of "younger" versus "older" sterile males in inducing egg sterility in large field enclosures at low and high overflooding ratios. As described below, the large enclosures contained multiple host trees, which created a physically complex environment that closely mimicked a natural setting. By introducing sterile males of a particular age (and competitive ability) with wild-like males and females, we directly tested whether, for young sterile males, the application of a high overflooding ratio compensated for low mating competitiveness and yielded a high level of egg sterility (i.e., comparable with that realized by older sterile males for the same high overflooding ratio).

## Materials and Methods

**Study Insects.** Owing to the limited availability of wild flies, we used flies from a recently established colony (hereafter referred to as REC flies) started with >500 adults reared from coffee, *Coffea arabica* L., berries collected on the island of Kauai. Pupae were sifted from vermiculite 7–12 d after fruit collection. Adults were held in screen cages and provided a sugar–protein mixture (3:1 by volume), water, and an oviposition substrate (perforated plastic vials containing a sponge soaked in lemon juice). Eggs were placed on larval medium over vermiculite for pupation. Adults used in the experiments were separated by sex within 1 d of emergence, well before reaching sexual maturity at 6–8 d and held in plastic buckets covered with nylon screening (volume 5 liters; 100–200 flies per bucket) at 23–27°C and 50–90% RH under natural and artificial light in a photoperiod of ≈12:12 (L:D) h. Experimental flies were supplied the same sugar–protein mixture as the colony. When used in experiments, male and female REC flies were 7–11 and 8–12 d old (i.e., sexually mature), respectively, and the colony was four to six generations removed from the wild.

Mass-reared flies were from a *tsl* strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, HI. Larvae of this strain were reared on standard diet, and males were dyed and irradiated 2 d before emergence at 150 Gy of gamma radiation from a $^{137}$Cs source. Sterile males used in the mating experiments were fed sugar agar (84.5% water, 14.7% sugar, 0.8% agar, and <0.1% methyl paraben [preservative]), but they were otherwise held in the same manner as the REC adults. For the experiments on induced egg sterility and mortality, we handled the flies following the protocol used at the California emergence facility. On the day of irradiation, we placed pupae in six paper bags (100 ml of pupae per

bag, where 1 ml is ≈60 males), which, in turn, were placed on the floor of a plastic storage box (a so-called PARC box, 60 by 48 by 33 cm [length:width:height] with screen panels on the lid and sides). Sugar agar was provided in 20- by 15- by 3-cm slabs placed on the screen on the top of the box. As described below, the timing of food placement (coincident with pupal placement or peak emergence) was varied among different treatments. At eclosion facilities, storage boxes are stacked to save space, and to mimic this situation, we placed empty boxes on top of the fly-containing boxes.

**Mating Experiments.** In the first experiment, we measured the mating propensity of sterile males in a noncompetitive environment. We placed 50 sterile males of ages 2, 3, 4, 5, or 10 d along with 50 virgin REC females in Plexiglas cages (30 by 30 by 40 cm) at 0830 hours, and we collected mating pairs over the next 3 h. On any given day, we ran four cages (two cages for each of two age groups selected haphazardly). Flies were used only once, i.e., each cage contained naïve virgin flies. Each age group was tested on five different days for a total of 10 replicates. For comparative purposes, we also measured the mating propensity of mature REC flies following the same protocol (50 REC males with 50 REC females per cage; 10 replicates run on five different days).

In the second experiment, we monitored the mating success of sterile males in competition with REC males for copulations with REC females. Mating tests were conducted at the USDA–ARS Laboratory, Honolulu, HI, in four standard outdoor field cages (3 m in diameter, 2.5 m in height) each containing two artificial trees (with leaves resembling those of *Ficus benjamina* L., 2.1 m in height). As flies perform all natural mating activities on these trees, using them eliminated the potentially confounding influence of plant chemistry on mating performance (e.g., Shelly and Villalobos 2004). Groups of 75 sterile males of a given age (same ages as described above, except that 2 d old males were omitted), 75 REC males, and 75 REC females were released in the cages between 0800 and 0830 hours (males were released 15 min before females), and mating pairs were collected over the next 3 h. Pairs were then chilled, and males were identified under a UV (black) light (sterile males, pink dye; REC males, no dye). In total, eight replicates were run for each age category. On most days, we ran two tents for two different age groups; consequently, replicates for a given age category were typically run on four different days. Air temperature ranged from 25 to 30°C during the trials.

The final experiment described age-related variation in male ability to inhibit female remating. Initial matings by REC females were obtained in the laboratory in Plexiglas cages. Approximately 200 females and 200 sterile males of a given age (same ages as described above, except 2-d-old males were again omitted) were placed in a cage between 0800 and 0830 hours, and mating pairs were collected continuously for 2–3 h. Only females from pairs coupled for >90 min were tested for remating; males were discarded. Mated females were held in plastic buckets and provided the sugar–protein mixture and water but no oviposition substrate. Females were given an opportunity to remate 2 d after the first mating. We placed 40 mated females and 40 REC males in Plexiglas cages (one cage = 1 replicate; $n = 10$ per male age group) at 0800 hours and collected mating pairs over the next 4 h. As in the first experiment (male mating propensity in a noncompetitive environment), we conducted an additional set of tests in the same manner as described above, except that REC males were used as first-maters to provide comparison with sterile males.

In addition to these laboratory tests, we ran a second set of remating trials in the field cages to gather comparative data under more natural conditions. These trials were conducted only for 3- and 10-d-old sterile males as well as for REC males (all as first maters). Initial matings were obtained in the laboratory following the above-mentioned procedures, but remating trials were run in field cages. Two days after the initial mating, 50 mated females and 50 REC males were released per tent between 0800 and 0830 hours, and mating pairs were collected over a 3-h period. Eight replicates (cages) were run for each male group.

**Effect of Storage Duration and Timing of Food Placement on the Mortality of Sterile Males.** As will be shown, the competitive mating study noted above revealed that sterile males ≥4 d old were superior competitors to 2-d-old sterile males. This result suggested that SIT programs that release sterile males at 2 d of age (e.g., California) might achieve greater effectiveness if sterile males were held two additional days before release. Consequently, we attempted to assess the effect on mortality (as operationally defined below) of holding sterile males in storage boxes for two extra days. Storage boxes were set up in the standard manner (≈36,000 males/box, food placement coincident with pupal placement) and placed in large screen cages (0.90 by 0.75 by 1.0 m). Room temperature was maintained at 25°C and 50–80% RH with lights on continuously. Then, the boxes were opened two or 4 d later (after removal of the empty box resting on the fly-containing box) by shifting the lid slightly off the corners to allow escape. Three days after opening the box, we collected all the flies (all dead) outside the box, dried them for 48 h, and then weighed them to the nearest 0.001 g. The number of flies was estimated through extrapolation with the known weight of dried males (0.5 g = 378.2 ± 1.9 [SE] flies; $n = 5$). Because the above-mentioned comparison showed a significant difference between boxes opened 2 or 4 d after pupal placement, we conducted another experiment in which boxes were opened 4 d after pupal placement but food was not placed on the boxes until the day of peak adult emergence (i.e., 2 d after pupal placement). In total, six replicates were completed for each of the three treatments (two boxes per treatment were run concurrently).

Regarding these tests, the method used to measure male mortality obviously allowed only approximate estimates. Although males outside the box clearly survived to release age, adult males inside the box may

have died before or after the box was opened. Males in the latter group may have been too weak to exit the box at all, or they may have reentered the box after exiting. If, however, we assume that flies too weak to exit were "dead" from the functional perspective of the SIT and that few "vigorous" flies reentered the box and/or that this number was relatively constant among treatments, and estimates of the numbers of exiting males allow, at least, a rough comparison of relative mortality levels among the different treatments.

**Induced Egg Sterility.** The ability of 2-d-old versus 4-d-old sterile males to induce egg sterility was compared using large field enclosures. The 2-d-old sterile males were handled in same manner described above for the Los Alamitos facility, i.e., the sugar-agar food was placed on the storage box at the time of pupal placement. For the 4-d-old males, however, the food was placed on the storage box on the day of peak emergence. Tests were conducted in four nylon-screen enclosures (16 by 6 by 2.5 m) set up in a guava, *Psidium guajava* L., orchard in Waimanalo, Oahu, HI. The tents contained 12–15 guava trees and were covered with shade cloth to reduce insolation. As described below, trials lasted 4 d, and they were run concurrently in two enclosures, testing 2-d-old and 4-d-old sterile males, respectively. Enclosure pairings were the same over the entire study, resulting in a use/nonuse schedule that alternated weekly for each pair of enclosures. Within each of the enclosure pairs, the age of sterile males released in a given enclosure was alternated between successive replicates.

We measured egg sterility for 2- and 4-d-old sterile males at overflooding ratios of 5:1 and 100:1. For the 5:1 ratio, we released 200 REC males, 200 REC females, and 1,111 sterile males of the appropriate age per enclosure. To obtain the sterile males, we removed one paper bag (and the males resting on it) from the storage box, quickly transferred it to a screen cage (30-cm cube), counted, and removed males by using an aspirator, and we placed them in plastic buckets for transportation to the field. Based on quality control data (California Department of Food and Agriculture, unpublished data), we estimated that 10% of eclosed sterile males were incapable of flight. To compensate for these individuals, we counted and released 1,111 sterile males (≈1,000 flight-capable males). For the 100:1 ratio, we released 288 REC males, 288 REC females, and ≈28,800 sterile males. For this ratio, males were released directly from storage boxes into the field enclosures. A volume of 100 ml of pupae (≈36,000 pupae) was placed in a storage box, and, assuming that 80% of these pupae yield males capable of flight (California Department of Food and Agriculture, unpublished data), the total number of flight-capable, sterile males released was ≈28,800.

The same schedule was followed for all trials. On day 1, food and water were introduced, and the flies were released. Food (sugar–protein mixture) was presented in petri dishes held within Jackson traps (lacking sticky inserts) suspended 1.5–2.25 above ground from tree branches at four evenly spaced locations. At each of these sites, we also provided water in a covered plastic cup (100-ml volume with an emerging cotton wick) held within a Jackson trap. The wires suspending the resource-laden Jackson traps from branches were coated with Tanglefoot (Tanglefoot Co., Grand Rapids, MI) to exclude ants. After the placement of food and water, males were released 20 min before females by placing the plastic buckets or the storage box in the center of an enclosure, removing the cover, and allowing males to fly away. The containers were tapped periodically to induce male flight, and ≈5 min before female release, the containers were inverted and tapped firmly to remove any remaining males. The male containers were the removed from the enclosure, and females were released from the center of the enclosure. Flies were released between 0900 and 1000 hours for all trials.

On day 3, 14 Granny Smith apples, *Malus domestica* Borkh., were placed in the enclosures at 1000 hours for oviposition. Apples were suspended 1.5–2.25 m above ground by piercing the fruit with a nail and connecting the nail to a branch with wire. Tanglefoot was applied to the wire to exclude ants. The apples served as the only available oviposition resource as guava fruits (if present) were removed before the start of each trial.

On day 4, apples were collected at 1000 hours, returned to the laboratory, examined for oviposition "sting" marks under a dissecting microscope, and eggs were removed using a scalpel and fine forceps. Eggs were placed on moistened blotter paper within petri dishes and then incubated at 27°C for 48 h. Hatch was then determined by reexamining the eggs under a dissecting microscope. For each trial, we also measured egg hatch of REC females mated exclusively to REC males in a field-cage over a single guava tree adjacent to the large enclosures. Two hundred individuals of each sex were introduced on day 1, two apples were introduced on day 2 for a 24-h period, and egg hatch was scored as described above.

After removal of the apples on day 4, we removed the food and water from the enclosures to hasten the death of flies from the just completed trial. As noted above, individual enclosures were used in alternate weeks, and few flies survived the week-long interval to the next trial. Nonetheless, before each release, we searched the enclosures and removed any surviving flies from the preceding trial. Eight replicates were performed per male age group per overflooding ratio, and eight replicates were performed per overflooding ratio for the small cage containing REC flies only.

**Statistical Analyses.** Comparisons among groups were made using raw data in analysis of variance (ANOVA) (*t*-test in two-sample cases) because assumptions of normality and equal variance were met in all instances. Where significant intergroup variation was detected, the Tukey test was performed to identify significant differences between group means. In tests of competitive mating ability, female remating tendency, and induced egg sterility, ANOVA was performed using arc sine transformed proportions. Means ± 1 SE are given.

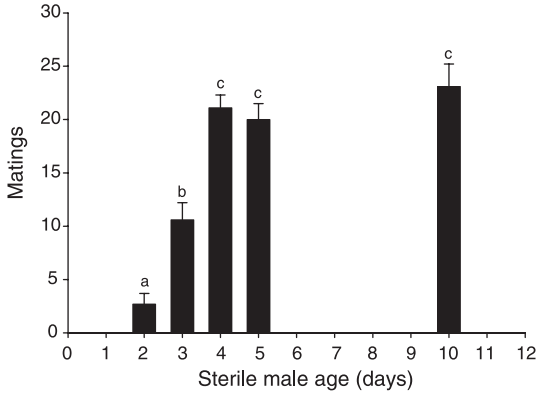


**Fig. 1.** Age-related variation in the mating propensity of sterile males in a noncompetitive environment. Bar heights represent means (±1 SE) recorded from laboratory cages ($n = 10$) containing 50 sterile males of a given age and 50 mature, virgin REC females. Means having different letters were significantly different by the Tukey test at the 0.05 significance level.

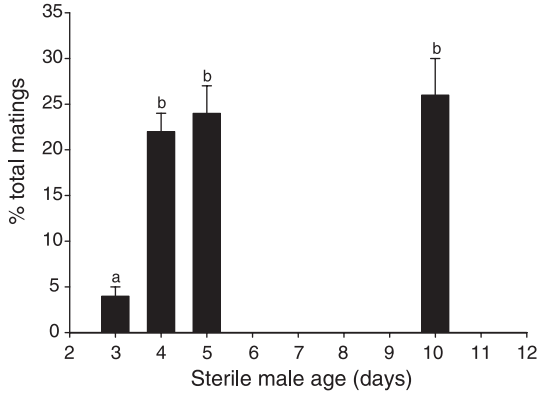


**Fig. 2.** Age-related variation in the relative mating success (% total matings) of sterile males in a competitive environment. Bar heights represent means (±1 SE) recorded from field cages ($n = 8$) containing 75 sterile males of a given age, 75 REC males, and 75 REC females. Means having different letters were significantly different by the Tukey test at the 0.05 significance level. Means based on raw data are presented, but the statistical analysis was performed using arcsine-transformed values.

## Results

**Mating Experiments.** In the noncompetitive experiment, there was significant variation in mating propensity among sterile males of different ages ($F_{4, 45} = 53.8$; $P < 0.001$) (Fig. 1). Among sterile males, mating activity increased dramatically over ages 2–4 d, and then it was relatively constant over ages 4–10 d. For example, there was approximately an eight-fold increase in mating pairs between 2- and 4-d old males (21.1/27 = 7.8), but only a 10% increase between 4- and 10-d-old males (23.1/21.1 = 1.1). In trials involving mature REC males, the number of mating pairs collected was, on average, significantly greater than that noted for the oldest (10 d) sterile males tested (39.2 ± 1.8 versus 23.1 ± 2.1, respectively; $t = 9.9$, $P < 0.001$).

Results from the competitive environment mirrored those of the noncompetitive tests. The total number of matings recorded per replicate did not vary significantly with the age of the sterile male being tested ($F_{3, 28} = 0.1$; $P > 0.05$), with an average of 36.8–38.8 total matings observed per replicate among tests involving sterile males of differing age. Consequently, we described the performance of sterile males using relative mating success (% total matings, Fig. 2). Relative mating success varied significantly with age of the sterile males ($F_{3, 28} = 15.7$; $P < 0.001$). The 3-d-old sterile males achieved only 4% of the total matings, a proportion significantly below that of all the other age categories. Among the 4-, 5-, and 10-d-old sterile males, relative mating success varied independently of age, and sterile males in these age categories obtained, on average, 22–26% of the total matings per replicate.

As measured in laboratory cages, there was significant age-dependent variation in the ability of sterile males to inhibit female remating ($F_{4, 50} = 34.9$; $P < 0.001$) (Fig. 3). On average, ≈62% (24.6/40) of REC females first mated to 3-d-old sterile males remated 2 d later compared with only ≈25% (10.8/40) of REC females first mated to 10 d old sterile males. In the laboratory cages, REC males were more successful at inhibiting female remating than sterile males of any age class (*t*-test; $P < 0.05$ in all cases), with only ≈10% (mean proportion = 4.3/40) of REC females remating after initially mating with a REC male.

Measurements of female remating in the field tents generated a similar trend. Female remating tendency varied significantly with respect to the identity of the first mating male ($F_{2, 21} = 5.8$; $P < 0.01$). On average, the proportion of remating females was significantly

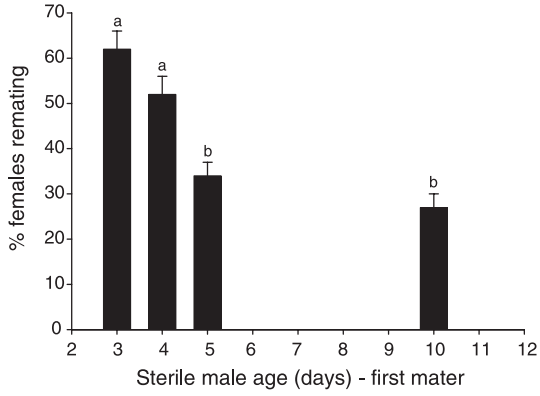


**Fig. 3.** Age-related variation in the ability of sterile males to inhibit female remating in laboratory cages. Bar heights represent mean proportion (±1 SE) of matings recorded from cages ($n = 10$) of 40 REC males and 40 REC females mated 2 d earlier to sterile males of a given age. Means having different letters were significantly different by the Tukey test at the 0.05 significance level. Means based on raw data are presented, but the statistical analysis was performed using arcsine-transformed values.

greater for females first mated to 3-d-old sterile males (16 ± 2.5%) than females mated to either 10-d-old sterile males (7.4 ± 1.2%) or REC males (5.9 ± 1.4%), and no difference in the frequency of female remating was detected between these latter two male groups (Tukey test; $P = 0.05$).

**Male Mortality in Storage Boxes.** Among boxes in which food placement was coincident with pupal placement, a significantly greater number of males exited boxes that were opened 2 d after peak emergence (27,438 ± 487; $n = 6$) than boxes opened 4 d after peak emergence (22,065 ± 378; $n = 6$) ($t = 3.9$, $P < 0.01$). There was no significant difference in the number of males that exited boxes that were opened 2 d after peak emergence with food placement coincident with pupal placement and the number that exited boxes opened 4 d after peak emergence with food placement delayed until the day of peak emergence (28,395 ± 720; $n = 6$) ($t = 1.2$, $P > 0.05$). Correspondingly, among boxes opened 4 d after peak emergence, the number of exiting flies was, on average, significantly higher for boxes receiving food on the day of peak emergence than those receiving food on the day of pupal placement ($t = 4.3$, $P < 0.01$).

**Induced Egg Sterility.** For individual replicates in the large enclosures, the total number of eggs collected from apples did not vary significantly among the different treatments ($F_{3, 31} = 1.7$; $P > 0.05$). For trials involving 2-d-old sterile males, the average numbers of eggs collected per replicate were 593 ± 97 and 730 ± 52 for the 5:1 and 100:1 overflooding ratios, respectively. For trials involving the 4-d-old sterile males, the average numbers of eggs collected per replicate were 567 ± 85 and 717 ± 83 for the 5:1 and 100:1 overflooding ratios, respectively. In the small cage containing REC flies only, we collected an average of 564 ± 56 eggs per replicate. Previously, we estimated an average daily egg output of ≈14 eggs per female (Shelly et al. 2005). Applying this value to the current study, we estimated that, on average, 40–52 females deposited eggs per replicate in the large enclosure (based on the minimum [567] and maximum [730] values for average total number of eggs collected per replicate). Similarly, we estimated that, on average, 40 females (564/14) deposited eggs in the small cage containing REC flies exclusively.

ANOVA performed using data from the large enclosures plus the small cage over all trials revealed significant variation in the level of egg sterility ($F_{5, 42} = 43.8$; $P < 0.001$) (Fig. 4). Interestingly, the level of egg sterility (33% unhatched eggs) observed for enclosures containing 2-d-old sterile males released at a 5:1 overflooding ratio did not differ significantly from the small cage containing REC flies exclusively (25 and 22% unhatched eggs, respectively, for replicates conducted during the two overflooding ratios). By contrast, in enclosures containing the 4-d-old sterile males at the 5:1 overflooding ratio, an average of 62% of the eggs collected did not hatch, a proportion significantly higher than that observed for the 2-d-old sterile males at the same overflooding ratio or that observed in the small cage with REC flies. For both age groups of sterile males, levels of egg sterility at the 100:1 overflooding ratio were significantly higher than those observed at the 5:1 ratio and those observed in the small field cages containing REC flies only. However, at this higher overflooding ratio, a significantly higher portion of unhatched eggs was noted, on average, for the 4-d-old (79%) than the 2-d-old (58%) sterile males. The incidence of egg sterility observed for 2-d-old sterile males at the 100:1 overflooding ratio did not differ significantly from that recorded for the 4-d-old sterile males at the 5:1 overflooding ratio.

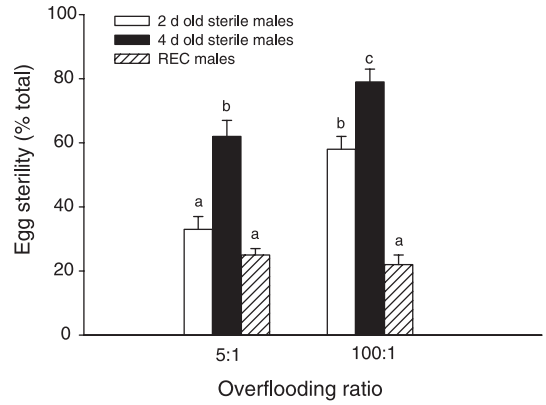


**Fig. 4.** Proportion of sterile eggs (% total) collected in large enclosures containing REC males and females and either 2-d-old or 4-d-old sterile males at 5:1 and 100:1 overflooding (sterile:REC males) ratios and in smaller field cages containing REC males and females only. Bar heights represent means (±1 SE). Means having different letters were significantly different by the Tukey test at the 0.05 significance level. Means based on raw data are presented, but the statistical analysis was performed using arcsine-transformed values. Eight replicates were conducted per age group per overflooding ratio in the large enclosures, and eight replicates were conducted per overflooding ratio for the small cage containing REC flies only.

## Discussion

The current study showed that, for the *tsl* strain investigated, 2- and 3-d-old sterile males had a lower mating propensity and lower mating competitiveness than 4-, 5-, or 10-d-old sterile males. In addition, although 4-d-old sterile males were competitively equivalent to the older sterile males tested, females first mated to 4-d-old sterile males displayed a higher tendency to remate (with a REC male) than did females first mated to 5- or 10-d-old sterile males. These data suggest that mating propensity and ability were low for young *tsl* males (<4 d old) and higher, and relatively uniform, among 4–10-d-old males. The finding that relative mating success was similar between 4- and 10-d-old sterile males indicated that mating competitiveness had already reached an asymptote and did not increase monotonically with male age.

Although failure to attain sexual maturity probably accounts for the low mating competitiveness observed for young males, it is less clear why older males would

be more successful in inhibiting female remating than younger males. Remating tendency in *C. capitata* females seems to vary inversely with the amount of sperm transferred during the initial mating. For example, Mossinson and Yuval (2003) found that females receptive to a second mating had, on average, <300 sperm in their spermathecae compared with 2,600 for unreceptive females. In addition, Miyatake et al. (1999) demonstrated that females mated to surgically castrated (i.e., spermless) males were more likely to remate than females mated to intact males. These findings suggest that *tsl* males ≥5 d old more effectively inhibit female remating, because they transfer more sperm than younger males. Although consistent with the aforementioned studies, this interpretation is counter to the results of Taylor et al. (2001), who reported an inverse relationship between male age and the amount of sperm transferred during copulation. Given this inconsistency, it seems that the age-dependent ability to inhibit female remating may reflect differences, not in sperm count, but in the composition or quantity of accessory gland secretions between young and old males (Jang 1995).

Independent of the underlying mechanisms, the present findings on age-dependent male performance differ noticeably from those presented for a bisexual strain mass reared in Metapa, Mexico. In field cage trials involving wild and mass-reared (irradiated) flies of variable ages, Liedo et al. (2002) found that, among sterile male–wild female pairings, 4-d-old sterile males accounted for approximately twice as many matings as all other male age groups combined. Thus, in the Mexican strain, male mating propensity seemed to rise and fall rapidly, generating a narrow peak of mating activity at 4 d of age. Based on this finding (and other tests involving mating among mass-reared flies exclusively), these authors concluded that male mating propensity for this mass-reared strain was maximal between 3 and 5 d of age and declined abruptly thereafter.

Because the *tsl* strain studied here and the Metapa strain were both maintained under standard mass-rearing conditions, the difference in the timing of male mating propensity likely reflects underlying genetic differences, which may favor different release strategies for the two strains. For the Metapa strain, Liedo et al. (2002) proposed that, given their rapid maturation, males would optimally be released at 1–2 d of age. These authors suggested that holding these males for longer prerelease periods would result in decreased mating propensity in the field, higher mortality in the storage boxes, and increased programmatic costs resulting from increased storage costs.

Although releasing the Metapa males at 1–2 d of age may be justifiable, the delayed sexual maturation reported here implies that implementing such an early release age for *tsl* males is inefficient, because the majority of males may die before attaining peak sexual activity. Consequently, we suggest that emergence facilities using *tsl* strains consider holding sterile males until they are 3–4 d old before release. This suggestion rests on the assumption that mortality after release exceeds that in storage. If postrelease mortality were lower or equal to prerelease mortality, then sterile males could be released at the earliest possible time, i.e., the timing of male release could occur independently of the timing of male maturation. However, several field studies indicate extremely high, postrelease mortality of sterile males of *C. capitata.* For example, in a mark–release–recapture study conducted in fruit growing areas in Mexico, Baker and Chan (1991) reported that nearly all of the trapped flies were caught within 2–3 d of release. Importantly, these authors present data indicating that this rapid disappearance was not the result of dispersal from the trapping grid and was thus likely the result of low survivorship (also see Wong et al. 1982; Shelly et al. 1994, 2006).

We recognize that extending the prerelease period would require a change to standard operating procedures regarding food placement. As shown, if the standard schedule of food placement was followed (i.e., coincident with pupal placement), holding sterile males for an extra 2 d (i.e., until 4 d of age) resulted in increased, prerelease mortality. However, if food placement was delayed (until the day of peak emergence), extending the holding time by 2 d did not lead to greater mortality. Given this finding, we recommend a temporal separation between pupal and food placement. Placing pupae and food at the same time is labor-saving, because processed boxes can be stacked and stored without further handling until knockdown and release. However, delaying food placement by 2 d would require "breaking down" stacks of boxes, applying the food, and then reconstructing the stacks. In our view, this increased work load would be more than offset by the increased mating competitiveness of 4-d-old males as evidenced by the egg sterility measurements obtained in the large field enclosures.

In conclusion, we urge caution in accepting a "quantity over quality" philosophy to guide SIT programs against the Mediterranean fruit fly. In our view, emergence facilities should not simply release flies at the youngest age possible to maximize the turnover rate of flies through the facility. To do so engenders the risk of releasing large numbers of young males, most of which may die before reaching sexual maturity. Rather, a release age should be selected that balances the risk of pre- and postrelease mortality with the timing of sexual maturation (see Abila et al. 2003 for a similar argument involving SIT against the tsetse fly). If males are not sexually competitive until 4–5 d of age and if males can be held without excessive cost (in terms of fly mortality as well as financial expense), then releasing smaller numbers of older males may actually be more effective than the converse.

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